

STRUCTURE OF THE MATURING TESTA OF *ACACIA GALPINII* BURTT DAVY

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ABSTRACT

The epidermal cells of the testa undergo anticlinal elongation during development. The cell walls consist of cellulose and pectic substances, whilst lignin is absent. Just before maturity, callose is deposited on the anticlinal walls of the epidermal cells. Large intercellular spaces are formed between adjacent cells of the columnar subepidermal hour-glass cell layer. Tylose-like bodies are present in these intercellular spaces.

UITTREKSEL

STRUKTUUR VAN DIE TESTA VAN *ACACIA GALPINII* BURTT DAVY TYDENS RYPING

Geurende ontwikkeling verleng die epidermale selle van die testa antiklinaal. Die wande bestaan uit sellulose en pektiese bestanddele, sonder lignien. Kort voor volwasenheid word kallose op die antiklinale wande van die epidermale selle neergelê. Groot intersellulêre ruimtes ontstaan tussen aangrensende kolomvormige selle van die subepidermale uurglas sellaa. Til-agtige liggame is in hierdie intersellulêre ruimtes teenwoordig.

1. INTRODUCTION

The testa of the Leguminosae seed has such a typical structure that fragments of material containing the external palisade-like epidermal cells and the underlying hour-glass cells may be recognized as belonging to this family (Corner, 1951). Seed structure within this family is, according to Corner, of fundamental importance in the classification of the members of the family, perhaps even to generic rank.

The structure of the seed of members of the genus *Acacia* may be used to differentiate between some of the subgenera and even some species (Watson, 1948; Vassal, 1963; Robbertse, 1973).

The work of Vassal (1975) has shown that relatively few differences exist between the structure of the testa of different *Acacia* species, although certain tendencies within this taxon may be elucidated by ratios calculated from cell size measurements.

The ontogeny of the testa appears to differ in detail only in different genera of the family. The work of Reeve (1946a, b) on *Pisum* and Sterling (1975) on *Phaseolus* reflects the basic similarities within the family.

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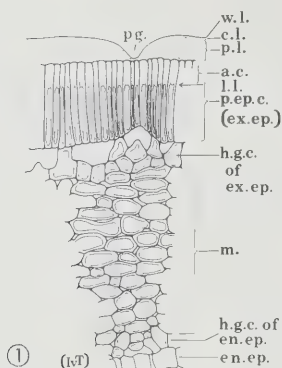


FIG. 1.

Diagram of a cross-section of a nearly mature testa. p.g.—pleurogram; w.l.—waxy layer; c.l.—cutinized layer; p.l.—pectic layer; a.c.—apical cap; l.l.—light line; p.ep.c.—palisade epidermal cells; ex.ep.—exo-epidermis; h.g.c.—hour-glass cells; m.—mesophyll; en.ep.—endo-epidermis.

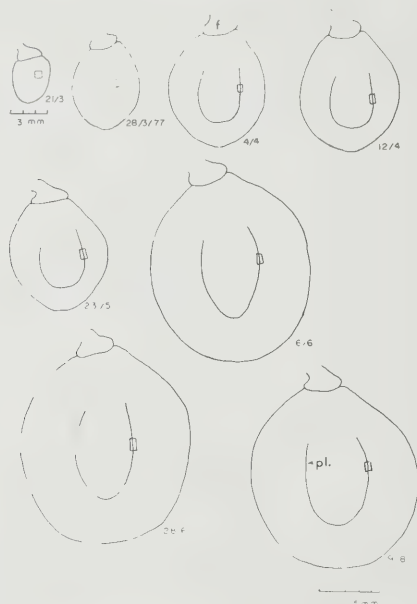


FIG. 2.

Size of seed at different ages and areas where testa was sampled. Dates when material was collected are indicated. pl.—pleurogram.

Acacia galpinii Burtt Davy was chosen for this study because the position of the light line in the testa does not vary appreciably in areas immediately adjacent to the pleurogram. The structure of these epidermal cells may therefore be regarded as being reasonably uniform for a considerable distance on both sides of the pleurogram. The structure and development of these cells are therefore less dependent on the exact area of the testa from which the studied material is obtained. A second reason is the slow development of the seeds, so that seed is available for study over an extended period.

The terminology used here is given in Figure 1 and is basically the same as that used by Corner (1951), with a few self-explanatory additions.

2. MATERIAL AND METHODS

All material was obtained from a stand of *Acacia galpinii* trees on the campus of the University of Pretoria. Flowers appear in August and the seeds reach a length of 1–2 mm in January of the following year. Fully mature seed is present in September or October.

Seeds were removed from pods, measured, and 1 mm wide strips of the testa, parallel to and including the pleurogram were cut out and placed in 6% glutaraldehyde in 0.05M Na-cacodylate buffer at pH 7.2. These strips were divided into lengths of 1.5 mm and fixed for a total period of 6 hours in the same solution. Post-fixation (2 h) was in 2% OsO₄ in the same buffer. Dehydration was done with ethanol or acetone with subsequent embedding in Spurr's (1969) medium. Figure 2 illustrates seeds at different ages as well as the position where samples of the testa were obtained.

Some duplicate batches of material were fixed and dehydrated at room temperature instead of 4 °C to compare the effect of temperature on the presence of microtubules, but no significant differences were found.

Thin (silver to gold) sections were cut with glass and diamond knives on a Reichert OMU 3 ultramicrotome. Sections were cut parallel to the long axes of the palisade epidermal cells, perpendicular to the pleurogram. Paradermal sections were cut from older material.

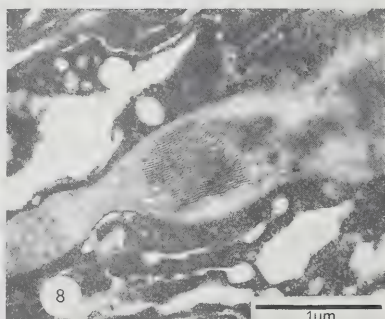
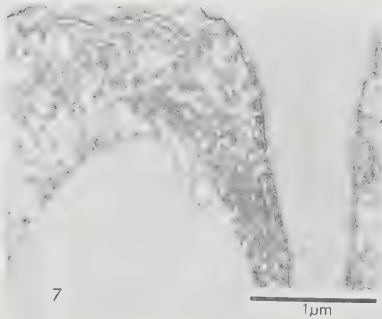
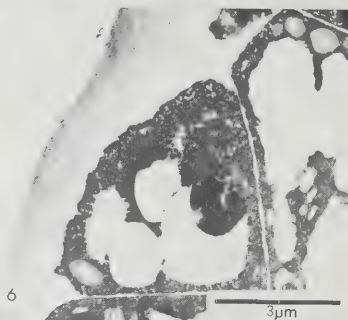
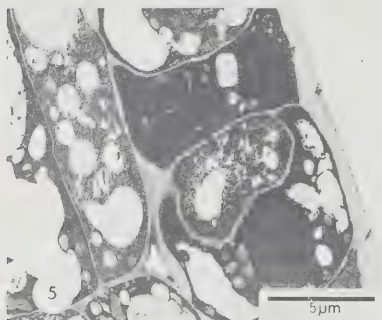
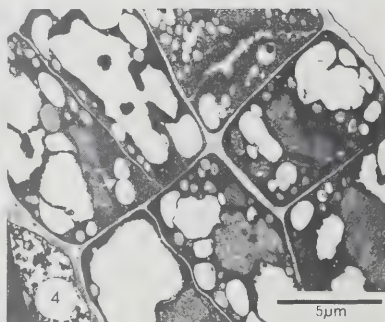
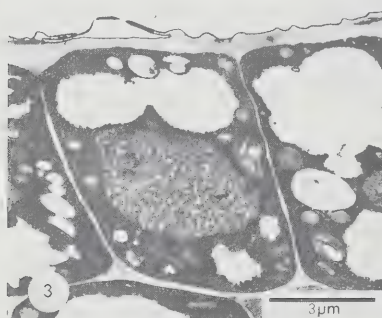
Contrast enhancement was either with potassium permanganate and lead citrate (Bray & Wagenaar, 1978) or with 4% uranyl acetate in 10% ethanol and lead citrate (Reynolds, 1963).

Sections (1–3 µm thick) of duplicate material fixed in buffered glutaraldehyde and embedded in glycol methacrylate (Feder & O'Brien, 1968) were cut with glass knives. These sections were stained with PAS-reagent (Nevalainen, Laitio & Lundgren, 1972) or with azure-methylene blue (Richardson, Jarret & Finke, 1960).

Histochemical tests were performed according to the standard procedures outlined by Jensen (1962). Some of the procedures used by Reeve (1946a) were

also applied. Representative sections were mounted in aniline blue (Smith & McCully, 1978) and examined with fluorescence optics.

Pieces of mature testa were mounted with epoxy adhesive on scanning electron microscope object carriers, broken or sectioned and sputter coated with 100 nm of gold. Fixation and critical point drying was found to be superfluous in the examination of the thick-walled epidermal cells at low magnification.



3. RESULTS AND DISCUSSION

The exo-epidermal cells of very young seeds (less than 3 mm long) have the general form of parenchyma cells although their outer walls may already be slightly thickened. Nuclei are situated centrally and large vacuoles are present near the inner and outer tangential walls of the cells. Small vacuoles are abundant and seem to be formed from centres very near to each other and to the large vacuoles because they often cause indentations of the membranes of adjacent vacuoles (Figs 3 & 4). This indentation may also be due to the fusion of small vacuoles to form fewer, large vacuoles. The cuticle is very thin and parts may flake off. The inner tangential walls begin to thicken at approximately this stage of development, while the radial walls remain thin until later. Due to convolutions of the radial walls, as well as to anticlinal divisions, the cells may assume unusual shapes (Figs 5 & 6). The outer cutinized part of the cell wall is laid down in layers parallel to the external surface.

When the seed is nearly 5 mm long, the exo-epidermal cells start to elongate. This elongation process continues until the seed is nearly mature and the typical palisade-like shape is reached. The formation of a system of longitudinal cell wall grooves and ridges is initiated at about the time when radial elongation of these cells commences. These grooves and ridges are responsible for the characteristic shape of the mature epidermal cells (Fig. 16).

Pectic substances which are added to the developing outer walls appear to shrink after deposition, with the result that the middle lamella (or regions corresponding to the position of the original middle lamella) assumes the shape of a wrinkled line. A few cells exhibit coarse lamellae of pectic materials alternating

FIG. 3.

Flaking cuticle on slightly thickened outer cell wall of 2 mm seed.

FIG. 4.

Vacuoles with indented membranes (arrowed) in exo-epidermal cells. Subepidermal cells exhibit newly formed periclinal cell walls. Seed 2,5 mm long.

FIG. 5.

Cell arrangement resulting from oblique anticlinal divisions. Seed 2,5 mm long.

FIG. 6.

Prismatic epidermal cell with distinct layering of wall material. Seed 3 mm long.

FIG. 7.

Apical part of exo-epidermal cell with dictyosomes and vesicles as well as rough endoplasmic reticulum orientated parallel to the developing cell wall. Seed 11 mm long.

FIG. 8.

Large, apparently inactive dictyosome in the basal part of an exo-epidermal cell from a 11 mm seed.

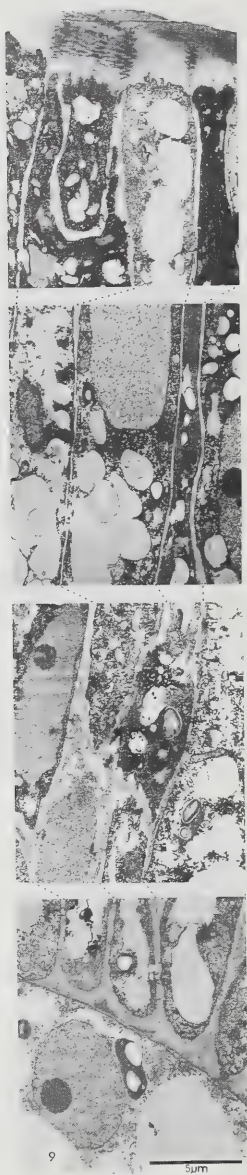


FIG. 9.

Composite electron micrograph of palisade epidermal cells from a 11 mm seed.

with presumably non-pectic substances (Fig. 9). Most cells, however, do not show these coarse, alternating layers and appear finely fibrillar, with fibrils arranged parallel to the outer surface.

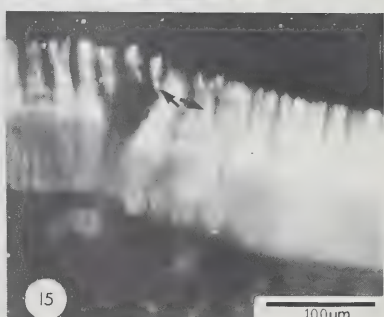
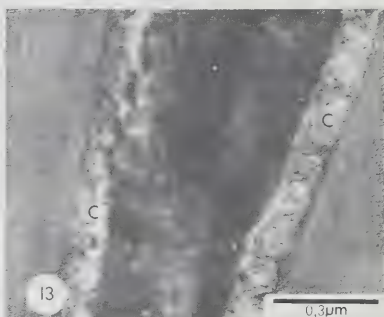
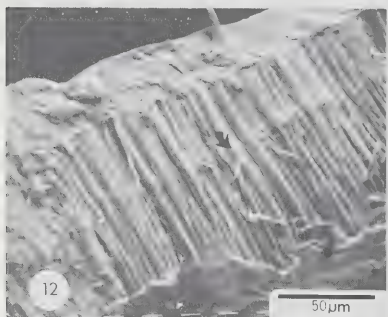
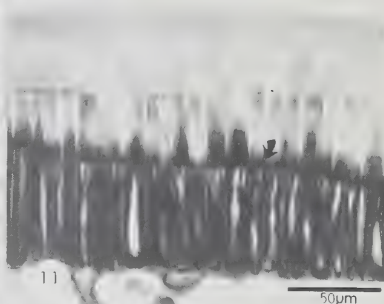
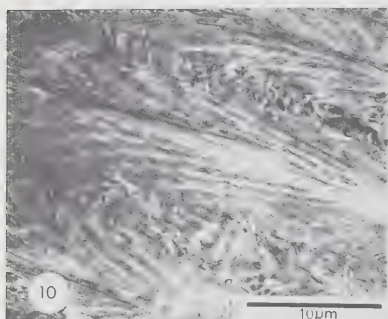
Large concentrations of sheets of rough endoplasmic reticulum are visible when the seed is approximately 11 mm long. These sheets are mostly arranged parallel to the developing walls. Whether this arrangement is of great importance, as Sterling (1975) implies, or whether it is a function of the fact that the cells are highly vacuolate with a thin layer of cytoplasm appressed to the cell walls, is not clear. Aggregations of polyribosomes and dictyosome cisternae with coated and uncoated vesicles are conspicuous near all developing cell walls, but are especially prominent near the apical ends of the exo-epidermal cells (Fig. 7). Many of these vesicles appear to coalesce with the plasmalemma. Dictyosomes consisting of approximately 30 closely appressed cisternae are occasionally present in the central or basal parts of these cells (Fig. 8). These dictyosomes do not appear to be active in secretion of vesicles, and the two faces of the complex are not nearly as well differentiated as that of the smaller, apically situated dictyosomes. These large dictyosomes are usually found close to the radial compartmenting walls.

Very few microtubules are visible at any stage in the development of the epidermal cells, as was also found by Sterling (1975) during his study of the formation of secondary cell walls of integuments in *Phaseolus*. Lipid bodies with multi-layered envelopes are present near the cell wall in most epidermal and mesophyll cells.

When the seeds reach a length of approximately 15 mm, size is no longer a reliable indication of maturity. Relatively large seeds may be less developed, when judging maturity by the thickness of epidermal and hour-glass cell walls, than appreciably smaller seeds.

When seeds are still less than 15 mm in length, the outer walls of the exo-epidermal cells have already undergone a great deal of thickening and (compare Fig. 1) consist of a thin, superficial waxy layer, a cutinized layer 3–5 μm thick, a pectic layer of nearly 10 μm and apical caps which may be 20 μm wide. The luminate basal parts of the cells may be a further 20 μm long. Ultimately, in fully mature seeds, the total length of the epidermal cells may be as much as 150 μm in the areas not directly associated with the pleurogram. This size is reached through continual increase in the thickness of all layers, except possibly the outermost cutinized layer. The cell wall material laid down in the apical caps is coarsely lamellar and is initially deposited nearly parallel to the middle lamella. Lamellae deposited at a slightly later stage are laid down in progressively more acute conical formations. The youngest parts of these cell walls which are deposited later and which occlude the lumina of the apical parts of the cells are apparently randomly arranged and not completely solid (Fig. 10) due to inclusions of probably cytoplasmic origin. According to Sterling (1975) the epidermal cell walls consist of nearly pure, highly crystalline cellulose in fibrous crystallites.

Histochemical tests, however, confirm the findings of Reeve (1946a) on the testa of *Pisum*, in that the apical ends of the cells test positive for pentosans. In this area of the wall, anticlinally orientated irregularities are present, which may be the same as the microchannels observed by Lyshede (1978) in the outer epidermal walls of stems of *Spartocytisus* of the Fabaceae. The irregularities observed here in the arrangement of the lamellar wall thickenings, are, however, not as clear as



those found by Lyshede and are visible only in electron micrographs. Typical microchannels should also be visible with the optical microscope, as implied by Hültsch (1966). These channels may be the route by which pectins and hemicellulose are deposited in the apical parts of the cell wall. The basal parts of the cells contain less of these substances. No lignin could be demonstrated by means of any of the usual tests in any cells of the testa, except in the vascular tissue. Cell wall material in these basal parts of the cells stain very deeply with the P.A.S.-procedure (Fig. 11) with the light line included in this darkly staining area. The position of the light line in this part of the testa is approximately in the middle or the upper third of the epidermal cells, as was also observed by Vassal (1975). When mature integuments are fractured and examined in the scanning electron microscope, the light line appears to be located at the position of a definite structural entity, consisting of a localized broadening of the cell wall (Fig. 12). This increase in width of the cells may be due to the measure of elasticity supplied to the cell walls by the presence of the flutes in the wall (Fig. 16). This may give rise to the refraction of light which is usually accepted to be the cause of this phenomenon, rather than the presence of a structural feature (Lute, 1928; Hamly, 1935; Corner, 1951).

Just before full maturity of the seeds is attained, large amounts of callose-like material are deposited onto (Fig. 13) and into (Fig. 14) the cell wall. This can also be seen when the cells are lightly stained with dilute aniline blue at physiological pH values and examined with fluorescence optics (Fig. 15). This appears to be in agreement with the findings of Reeve (1946a) who showed the presence of

FIG. 10.

Coarsely lamellar nature of cell wall thickenings initially laid down approximately parallel to the middle lamella and later assuming the characteristic conical shape. Seed 11 mm long.

FIG. 11.

P.A.S.-stained section of testa from 11 mm seed, showing the position of the light line (arrowed).

FIG. 12.

Fractured mature testa, illustrating the localized broadening of the cell walls at the position of the light line (arrowed).

FIG. 13.

Callose-like material (C), deposited on the cell wall of nearly mature exo-epidermal cells as seen in longitudinal section.

FIG. 14.

Callose-like material (C) deposited in the wall of exo-epidermal cells of a nearly fully mature testa. Paradermal section.

FIG. 15.

Fluorescence of callose (arrowed) in the apical parts of mature exo-epidermal cells.

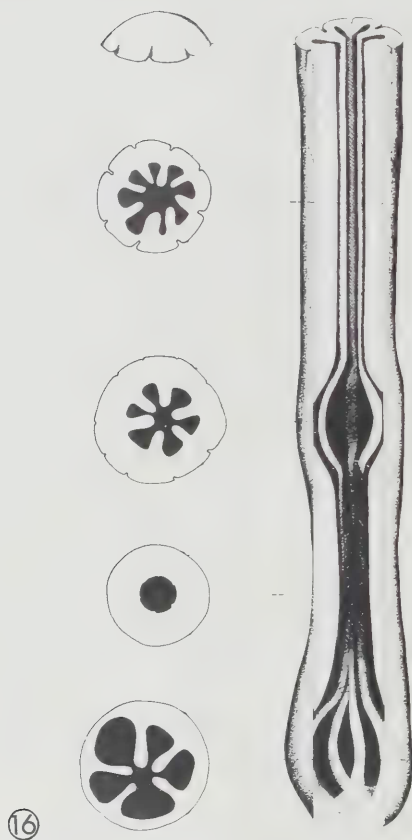


FIG. 16.

The structure of a mature epidermal cell, excluding all layers external to the apical cap.

"encrusting pentosan materials", especially in the light line area and in the middle lamellae of fully mature integuments of *Pisum*.

Tannin deposits are present in nearly all mature cells of the testa. The time of initiation of such deposits is, however, very variable. Some seeds contain appreciable amounts of tannin when the seed is less than 4 mm long. In other cases, seeds as large as 14 mm show little evidence of such deposition. Tannin is, however, nearly always present in exo-epidermal cells of seeds longer than 5 mm.

Up to the time when the cells of the exo-epidermis start to elongate, the development of the endo-epidermal cells proceeds along very similar lines. At later stages of development, the endo-epidermal cells increase in width by means of cell enlargement. Anticlinal divisions occur in a few of the cells. The endo-epidermal cells initially remain thin walled and never elongate periclinally to form palisade-like cells such as the exo-epidermis. At about the 11 mm seed size, some radial walls of these cells appear to be perforate (Fig. 17). Whether this is due to localized lysis of the cell wall, or to the ingrowth of the walls, is not clear. Due to the pressure of the developing cotyledons, the anticlinal walls are folded. These folds are supplemented by localized growth of these anticlinal walls (Figs 18 & 19).

At maturity the first subepidermal cell layer below each epidermis shows the typical columnar structure as well as large intercellular spaces. These cells are basally and apically wider than in the centre, so that the term hour-glass cell, is very appropriate. A survey of the literature shows that both the terms "hour-glass cells" and "osteosclereids" are freely applied to these cells. With regard to the complete absence of lignin from the walls of these cells and the characteristic shape, peculiar to the testa of the Leguminosae, the name hour-glass cells should perhaps be given preference.

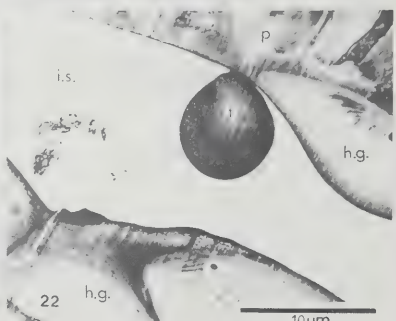
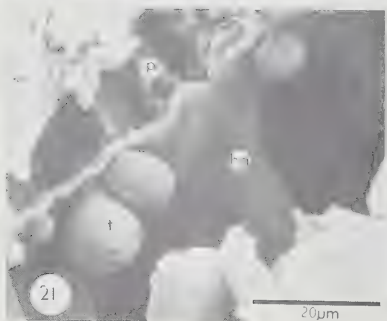
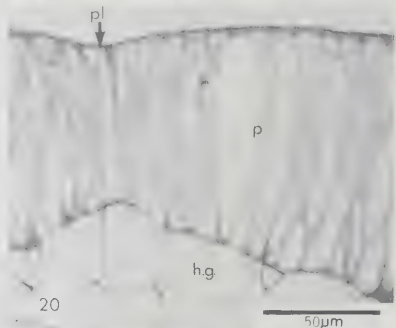
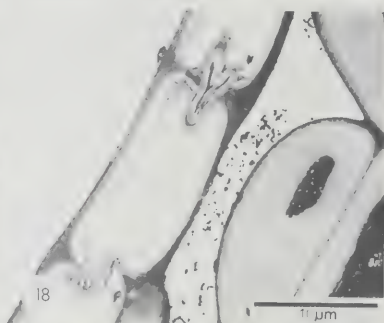
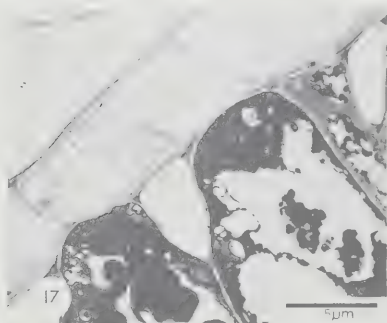
The hour-glass cells are initially thin walled and divide periclinally (Fig. 20). Anticlinal divisions occur very seldom except in the area immediately below the pleurogram. It appears as if this subepidermal hour-glass cell layer keeps pace with the increase in seed size by means of lateral stretching. This leads to tension in this layer, which can, in this case, only be relieved by the formation of large intercellular spaces. These spaces start to appear when the seed is ca. 5 mm long. The development of the hour-glass cells of the endo-epidermis proceeds along similar lines to those of the exo-epidermis, but these cells are not as extremely adapted.

This degree of development of the hour-glass cell layers is typical of the subgenus *Aculeiferum* (Vassal, 1975) or, according to Robbertse (1973), the subgenus *Vulgares*.

The development of the hour-glass cells is essentially complete when the seeds are about 20 mm long but still not mature. Intercellular deposits of tannin may be present (Fig. 18) in the cavities adjoining the epidermal cells.

Tylose-like bodies appear to be extruded from the basal parts of the exo-

epidermal cells and are present in the intercellular spaces between the epidermal and hour-glass cells (Figs 21 & 22). These bodies contain significantly more silicon than adjacent cells (as monitored by microprobe X-ray analysis). If the epidermal cell contents are under pressure, while a low pressure area exists in the intercellular spaces of the hour-glass cells, such tylose-like extrusions could be formed when thin areas of the basal cell walls of exo-epidermal cells bulge outwards.



None of the sections examined, however, showed continuity between the cytoplasm of the epidermal cells and presumed cytoplasmic inclusions of the tylose-like bodies.

The pleurogram is visible in immature seeds as a localized area where the exo-epidermal cells are shorter than the cells from other areas (Fig. 20). The area at the base of these shorter cells are filled in by cells formed by periclinal divisions in the young subepidermal hour-glass cells. In mature seeds the groove thus formed is made more prominent by the deposition of the pectic layer in areas lateral to the pleurogram.

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FIG. 17.

Perforate radial walls of endo-epidermis in a 11 mm seed.

FIG. 18.

Folded anticlinal walls of the endo-epidermis, with tannin in the intercellular spaces between hour-glass cells.

FIG. 19.

Result of pressure and growth on the anticlinal walls of endo-epidermal cells from a 19 mm seed.

FIG. 20.

Thin walled hour-glass cells (h.g.) and palisade epidermal cells (p) of a 8 mm seed, sectioned through the pleurogram (pl).

FIG. 21.

SEM micrograph showing tylose-like bodies (t), hour-glass cells (h.g.) and palisade epidermal cells (p) in a mature seed.

FIG. 22.

Electron micrograph of a section through a tylose-like body of a 20 mm seed. i.s.—intercellular space; t—tylose-like body; h.g.—hour-glass cell; p—palisade epidermal cell.

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